Explor Anim Med Res,
Vol.10, Issue - 2, 2020, p. 124-133

ISSN 2277- 470X (Print), ISSN 2319-247X (Online)
Website: www.animalmedicalresearch.org

Research Article

SYNTHESIS AND *IN VITRO* ASSESSMENT OF ZINC OXIDE NANOPARTICLES FOR THEIR ANTIOXIDANT AND ANTIBACTERIAL POTENTIALS

Vijayta Gupta [1*], Vinay Kant [2], Meena Sharma [1]

Received 20 December 2019, revised 29 July 2020

ABSTRACT: Nanoparticles have emerged as wonders of medicine. Zinc oxide (ZnO) nanoparticles have received much attention and are in the forefront of research. In present study, ZnO nanoparticles were synthesized by chemical method and they were *in vitro* evaluated for antioxidant and antimicrobial potentials. The suspension of different concentrations of ZnO nanoparticles were evaluated for different parameters on day 1, 30 and 60 after its preparations. The average size of ZnO nanoparticles synthesized in this study was in the range of 73-87 nm with a polydispersity index of 0.578. ZnO nanoparticles scavenge different *in vitro* free radicals (ABTS, DPPH, superoxide anion and hydrogen peroxide) in concentration dependent manner. The percent inhibitions and IC_{50} values of ZnO nanoparticles against free radicals were more and less, respectively on day 1 as compared to day 30 and 60. The antioxidant and antibacterial actions of ZnO nanoparticles suspensions was lower on day 60 as compared to day 1. MIC and MBC values of ZnO nanoparticles on day 1 against *S. aureus* were 8.0 and 64.0 µg/ml, respectively. MIC and MBC values of ZnO nanoparticles on day 1 against *E. coli* were 32.0 and 256.0 µg/ml, respectively. MIC and MBC values against both bacterial strains were increased on storage of ZnO nanoparticles for 60 days. In conclusions, the use of ZnO nanoparticles can be extended in the field of Veterinary sciences, Medical sciences, agriculture, food sciences, tap water and wastewater disinfection etc. to counter the diseases/disorders/problems associated with free radicals and bacteria.

Key words: Zinc oxide nanoparticles, *In vitro* potentials, Antioxidant, Antibacterial, MIC, MBC.

INTRODUCTION

Nanotechnology has emerged as one of the most promising areas of research in science and technology, which has brought a paradigm shift in life sciences and revolutionary transformation in domains ranging from health to industrial materials. It signifies innovation and platform to make novel nanomaterials for a wide range of biological and biomedical applications (Singh *et al*. 2014). This technology has also emerged as a more impactful and rapidly developing technology in several other fields of human life as well, i.e. optics, chemical sensors, electronics, solar cell storage, food packaging and cosmetics, all over the world (Panda *et al*. 2017, Sangeetha *et al*. 2011). Nanoparticles are the key product of nanotechnology. Different types of nanoparticles or nanomaterials are used in consumer goods in day-to-day life and these are synthesized by using physical, chemical, or biological methods for several applications. Recently, nanoparticles have been considered as wonders of medicine. Nanoparticles are seemed more impressive as they have unique physical properties, i.e. relatively small size, higher surface energy, and optical, magnetic, chemical and biological behaviors (Rehana *et al*. 2017, Vijayakumar *et al*. 2016). The efficiency of action of nanoparticles is not only depends on the nanoparticle's concentration, but size of nanoparticles also has great influence (Ankanna and Savithramma 2011, Qi *et al*. 2004).

Increased production of highly reactive oxygen species (ROS) in biological system leads to oxidative stress (Nita and Grzybowski 2016), which has been observed to increase the incidences of different diseases such as cancer, cardiovascular disease, diabetes, arthritis, skin problems by UV rays etc. (Nash and Ahmed 2015).

[1]Department of Chemistry, University of Jammu, Jammu, (J&K)-180006, India. [2]Division of Pharmacology, IVRI, Izatnagar, U.P. Present address: Department of Veterinary Pharmacology & Toxicology, LUVAS, Hisar, 125004, Haryana, India.
Corresponding author. e-mail: vijayta1gupta@gmail.com

Different antioxidants play important roles in neutralizing the actions of different free radicals in the biosystems. In recent years, bacterial resistance has also emerged very rapidly and become as one of the major concerns of the 21st century at global level. Different approaches involving the nanotechnology for several different metals and metal oxides have attempted with some good results in respect to their potent antioxidant and antibacterial actions (Yaqoob *et al*. 2020, Tettey and Shin 2019).

Zinc oxide (ZnO), one of the metal oxides, possesses several biological actions and its nanoparticles forms have better potentials. ZnO has been considered "generally recognized as safe" (GRAS) for use by the U.S. Food and Drug Administration (21CFR182.8991), and its nanomaterials have low toxicity and optimum biodegradability (Pati *et al*. 2014, Ko *et al*. 2018). In the recent past, ZnO nanomaterials have received much attention and are in the forefront of research due to their unique properties as well as wide applications. Different biological potentials and applications of ZnO nanoparticles include antibacterial, antifungal, anti-inflammatory, wound healing, anti-corrosive, UV filtering, antioxidant, anticancer, anti-dandruff etc. (Pati *et al*. 2014, Ratney and David 2017, Jamdagni *et al*. 2018).

Although, antioxidant and antibacterial activities of ZnO nanoparticles prepared by different approaches have been evaluated in some studies, but the studies related to the stability of prepared nanoparticles and their antioxidant as well as antibacterial potentials after their storage for different durations are completely lacking to the best of our knowledge. So, in present study, we synthesized the ZnO nanoparticles for the evaluation of their antioxidant and antibacterial activities on day 1, 30 and 60 to determine their stability and antioxidant and antibacterial potentials during its storage for different durations.

MATERIALS AND METHODS

Chemicals used

Zinc acetate, zinc oxide, sodium hydroxide, dimethyl sulfoxide (DMSO), ascorbic acid, 2,2-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS), potassium persulfate, 2,2- diphenyl-1-picryl hydrazyl (DPPH), phenazine methosulphate (PMS), nitroblue tetrazolium (NBT), hydrogen peroxide (H_2O_2), ferric chloride ($FeCl_3$), nicotineamide adenine dinucleotide hydrogen salt (NADH), disodium hydrogen phosphate, potassium dihydrogen phosphate, 2- deoxy-D-ribose, potassium dihydrogen phosphate (KH_2PO_4), potassium hydroxide (KOH), ethylene diamine tetramine (EDTA), nutrient agar (NA), nutrient broth (NB) etc. of analytical grade were purchased from Sisco Research Laboratories (SRL), New Delhi, India. The *Staphylococcus aureus* (*S. aureus*) (MTCC 1430) and *Escherichia coli* (*E. coli*) (MTCC 2127) were two bacterial strains gifted from the Department of Biotechnology, University of Jammu and used in this study. Tetracycline and gentamicin were used as standard antibiotics and procured from Sigma Aldrich, USA.

Synthesis of ZnO nanoparticles

Nanoparticles of ZnO were synthesized by using zinc acetate [$Zn(CH_3COO)_2.2H_2O$] and sodium hydroxide as precursor chemicals. Briefly, aqueous solution of zinc acetate (0.5 M) was kept under on magnetic stirrer at 80 °C for one hour and NaOH aqueous solution (2.5 M) was added drop by drop under high speed constant stirring after complete dissolution of zinc acetate. After 2 hrs of the completion of reaction, the solution was kept overnight, and the supernatant solution was separated carefully on next day. The precipitates from remaining solution were removed by using centrifuge (Make: Remi Lab World, India) at 6000 rpm speed for 10 min. Precipitated ZnO nanoparticles were washed and dried in oven at about 60 °C. The size of synthesized ZnO nanoparticles was determined within a day by Zetasizer (Make: Malvern Instruments, USA). The ZnO nanoparticles were further evaluated for antioxidant potentials against different *in vitro* free radicals (ABTS, DPPH, superoxide anion and hydrogen peroxide radicals). The *in vitro* minimal inhibitory concentrations (MIC) and minimal bactericidal concentrations (MBC) of synthesized ZnO nanoparticles were determined against *S. aureus* and *E. coli* bacterial strains by using broth dilution assay and two-fold dilutions of nanoparticles were used (Humberto *et al*. 2010). The suspensions of ZnO nanoparticles were kept at room temperature for maximum 60 days. The average size of nanoparticles was again determined on day 30 and 60, and they were also again evaluated for the different *in vitro* parameters on day 30 and 60.

Antioxidant activity of ZnO nanoparticles

Different concentrations of ZnO nanoparticles (6.25 to 800.0 µg/ml) were used to determine the total antioxidant activity based on $ABTS^{\bullet+}$ scavenging assay according to the method of Re *et al.* (1999) and Kant *et al.* (2012). The ABTS radicals scavenging capacity was performed by mixing different concentrations of ZnO nanoparticles with $ABTS^{\bullet+}$ solution. After proper mixing, the absorbance was recorded at 734 nm after 3 min. $ABTS^{\bullet+}$ solution without nanoparticles was used as

control solution. The ascorbic acid was used as standard antioxidant. The percentage of inhibition of $ABTS^{\bullet+}$ radicals at different concentrations were determined by using the following formulae:

% $ABTS^{\bullet+}$ inhibition = [1 – (A_{734}nm Sample/ A_{734}nm Control)] x 100

Different concentrations of ZnO nanoparticles (6.25 to 800.0 µg/ml) were used to determine the free radical scavenging activity based on DPPH radicals scavenging assay according to the method of Hsu *et al.* (2006) and Kant *et al.* (2012). DPPH radical scavenging assay is an easy and fast way to screen the antioxidant activity of the test compound (Huang *et al.* 2005). DPPH is a stable compound and accepts hydrogen or electrons (Gulcin 2004). By getting hydrogen or electron from donor atom DPPH free radical is reduced (Bhakya *et al.* 2015). The odd electron of DPPH accepts the hydrogen atom from the antioxidants and changes to identical hydrazine (Contreras-Guzman and Strong 1982). Deep violet color of DPPH solution gradually changes to pale yellow in the presence of antioxidants. The DPPH radicals scavenging capacity was performed by mixing different concentrations of ZnO nanoparticles with DPPH solution. DPPH solution without ZnO nanoparticles was used as control solution. Solutions were kept at room temperature (22 ± 3°C) in the dark for 30 minutes and the absorbance of the samples and control solutions were determined at 517 nm. The ascorbic acid was used as standard antioxidant. The % DPPH radical scavenging activity was calculated as follows:

% DPPH radical scavenging activity = [1– (A_{517}nm sample / A_{517}nm control)] x 100

Superoxide anion radicals scavenging ability of different concentrations of ZnO nanoparticles (6.25 to 800.0 µg/ml) was determined by the method described by Nishikimi *et al.* (1972) and Kant *et al.* (2012). The reaction mixture contained 1 ml of NBT solution (156 µM), 1ml of NADH solution (468 µM) and 0.5 ml diluted sample of different concentrations fraction. Addition of 100 µL PMS solution (60 µM prepared in phosphate buffer, pH 7.4) to the mixture was done to accelerate the reaction. The reaction mixture and control sample (without nanoparticles) were mixed properly and incubated at 25^0C for 5 min and absorbance at 560 nm was measured. The ascorbic acid was used as standard antioxidant. Percentage inhibition of the superoxide anion radicals was calculated using the following equation:

% superoxide radical scavenging activity = [1– (A_{560}nm sample / A_{560}nm control)] x 100

Different concentrations of ZnO nanoparticles (6.25 to 800.0 µg/ml) were used to determine the scavenging of hydrogen peroxide radicals according to the method of Jayaprakasha *et al.* (2004). A solution of hydrogen peroxide (20 mM) was prepared in phosphate buffered saline (pH 7.4). One ml of various concentrations of ZnO nanoparticles and ascorbic acid (standard antioxidant) were added to 2 ml of hydrogen peroxide solution in PBS. After 10 min, the absorbance was measured at 230 nm. Percentage inhibition of the hydrogen peroxide radicals was calculated using the following equation:

% Hydrogen peroxide radical scavenging activity = [1– (A_{230}nm sample / A_{230}nm control)] x 100

Table 1. IC_{50} values (µg/ml) of ZnO nanoparticles against ABTS, DPPH, superoxide anion and hydrogen peroxide radicals on different days.

Parameters	Ascorbic acid	ZnO nanoparticles		
		Day 1	Day 30	Day 60
Total antioxidant activity (ABTS) (µg/ml)	79.13±1.75	208.67±1.46^{a}	261.10±2.16^{ab}	286.74±2.02^{abc}
Free radical scavenging activity (DPPH) (µg/ml)	63.36±2.16	229.67±1.66^{a}	323.53±1.21^{ab}	381.45±0.86^{abc}
Superoxide radical scavenging activity (µg/ml)	82.11±3.18	336.31±1.08^{a}	383.85±1.39^{ab}	429.37±1.13^{abc}
Reducing power activity (µg/ml)	86.51±3.46	273.46±1.27^{a}	311.20±1.56^{ab}	374.72±1.06^{abc}

Data are expressed as means ± SE. $^{a, b, c,}$ represent $p < 0.05$ vs ascorbic acid, ZnO nanoparticles day 1 and ZnO nanoparticles day 30, respectively on the same day.

Table 2. The MIC and MBC values (µg/ml) of ZnO nanoparticles (NPs), tetracycline and gentamicin against *S. aureus* and *E. coli* .

Bacteria	ZnO NPs	ZnO NPs (Day 1)	ZnO NPs (Day 30)	Tetracycline (Day 60)	Gentamicin
			MIC		
S. aureus	8.0	8.0	16.0	4.0	2.0
E. coli	32.0	32.0	32.0	2.0	0.5
			MBC		
S. aureus	64.0	64.0	128.0	20.0	16.0
E. coli	256.0	256.0	512.0	10.0	8.0

Antibacterial activity of ZnO nanoparticles

Minimal inhibitory concentrations (MIC) of ZnO nanoparticles were determined against *S. aureus* and *E. coli* bacterial strains by using broth dilution assay and two-fold dilutions of nanoparticles were used (Humberto *et al*. 2010). The overnight incubated suspensions of both the bacterial strains were aseptically inoculated (about 10^7 CFU/ml) separately to 15 ml tube nutrient broth medium. Ten different dilutions of ZnO nanoparticles were prepared (2, 4, 8, 16, 32, 64, 128, 256, 512 and 1024 µg/ml) in DMSO were used and tests were performed in triplicates for each concentration for each bacterial strain. DMSO alone was also used as control for this study. The inoculated sets were incubated at 37ºC overnight. After incubation period, the visible turbidity in each tube was detected by the unaided eye (CLSI 2012). The lowest concentration with no turbidity was considered as the MIC for the tested strain. The minimum bactericidal concentrations (MBCs) of ZnO nanoparticles were determined by sub culturing the contents of nutrient broth used for MIC tests on nutrient agar plates (Ewnetu *et al*. 2013). The plates were incubated at 37ºC to see bacterial growth after 24 hours incubation. The concentration that showed no growth was considered as the MBC for the tested bacterial strain.

Statistical Analysis

Results are expressed as Mean ± SE with n equal to the number of replicates. The statistical significance was analyzed by applying two-way analysis of variance (ANOVA) followed by Bonferroni's post test using the GraphPad Prism v4.03 software program (San Diego, CA, USA). The differences between the different treatments were considered statistically significant at $p < 0.05$ or lower.

RESULTS AND DISCUSSIONS

The average size of ZnO nanoparticles synthesized in this study was 73-87 nm with a polydispersity index of 0.578. The average size of nanoparticles in the suspension on day 30 and 60 was 155-174 nm and 240-263 nm, respectively. The increase in size of nanoparticles on storage was might be due to the agglomeration of particles. In our earlier study, ZnO nanoparticles synthesized by the same method were spherical shape and average size by transmission electron microscopy (TEM) studies was in the range of 28-43 nm (Gupta *et al*. 2016). The variations in size of nanoparticles were might be due to accuracy of the instruments and sample lot differences. It has been observed that small size of the nanoparticles allows their internalization into cells, and to interact with biomolecules within or on the cell surface, enabling them potentially to affect cellular responses in a dynamic and selective manner that makes them well suited for biological applications (Cho *et al*. 2008).

Many disorders or diseases in humans and animals associated with the free radicals and the free radicals have been found accountable for producing cancer, diabetes, cardiovascular disease, ulcerative colitis, aging, atherosclerosis, Alzheimer's disease, neural disorders, mild cognitive impairment, Parkinson's disease, alcohol induced liver disease etc. (Velavan 2011). There are different *in vitro* methods which are used to determine the antioxidant activities. However, the results obtained from *in vitro* methods are difficult to apply to biological systems and do not inevitably predict a similar *in vivo* antioxidant activity. Different *in vitro* methods that have been developed to assess the antioxidant activity have strengths as well as limitations and a single measurement of antioxidant capacity usually is not sufficient. Thus, a number of different methods may be necessary to adequately assess *in vitro* antioxidant activity of a specific

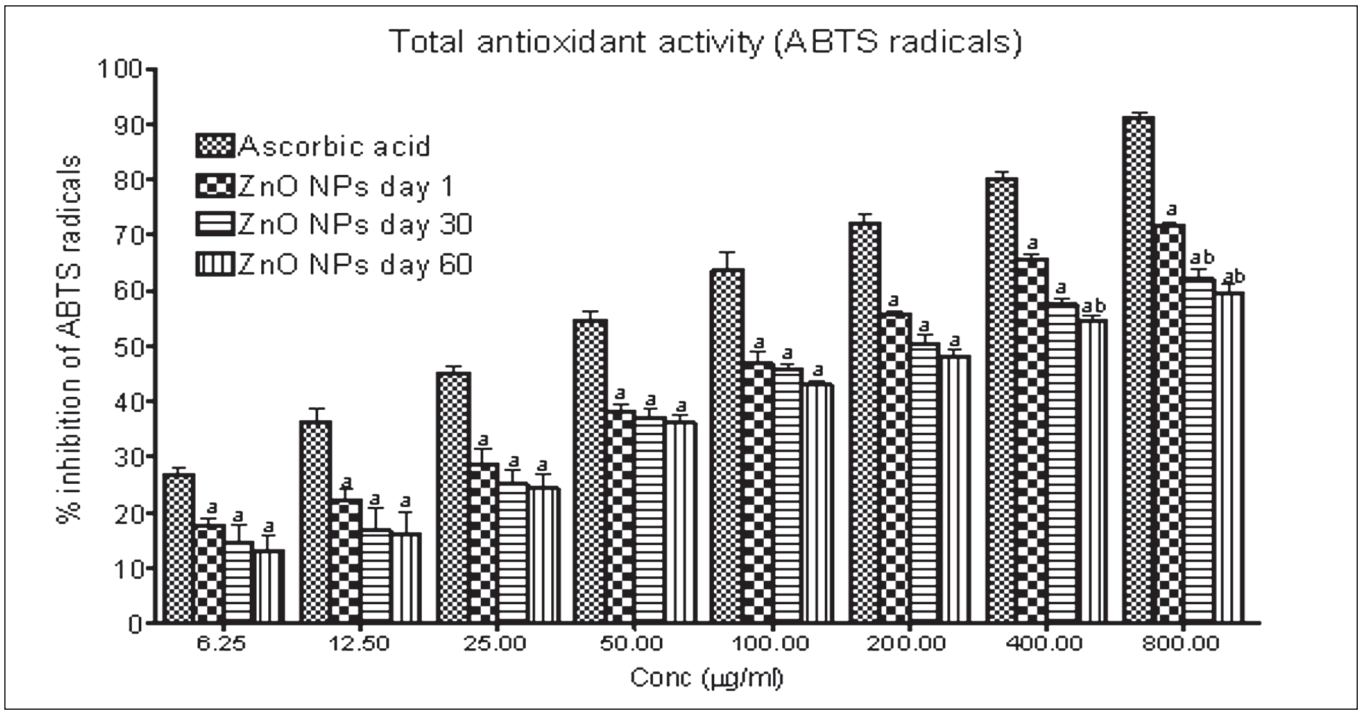

Fig. 1. Total antioxidant activity (ABTS radicals scavenging) of different concentrations of ZnO nanoparticles (NPs) on days 1, 30 and 60.

Results are expressed as Mean ± SE (n = 3). [a, b, c,] represent $p < 0.05$ vs ascorbic acid, ZnO nanoparticles day 1 and ZnO nanoparticles day 30, respectively at the same concentration.

compound. In present study, different concentrations of the synthesized ZnO nanoparticles were investigated using ABTS, DPPH, superoxide and hydrogen peroxide radical scavenging assays with respect to the standard antioxidant *i.e.*, ascorbic acid.

ABTS radical scavenging is a more sensitive and specific method to predict the free radical scavenging effect of any test compound (Ilhami *et al.* 2010). ABTS radicals are chemically produced, and their stability in a wide pH range and solubility in both the organic as well as aqueous media has raised the interest in the use of ABTS radical for the screening of the antioxidant activity of the test compound (Bala *et al.* 2015). In present study, synthesized ZnO nanoparticles and ascorbic acid (standard antioxidant) at different concentrations showed the inhibition of ABTS free radicals in a concentration dependent manner on different days (Fig. 1). The ABTS radicals scavenging potentials of ZnO nanoparticles were significantly lowered at all the concentrations after their storage for 30 and 60 days and this was might be due to agglomeration of particles (Bruinink *et al.* 2015, Zakharova *et al.* 2015). On day 1, the IC_{50} values (Table 1) of ZnO nanoparticles against ABTS radicals were significantly lower than the values of day 30 and 60. This revealed that IC_{50} value of ZnO nanoparticles for ABTS radicals significantly increased on storage and all these values were also significantly lower than ascorbic acid. It is known that lower the IC_{50} values greater the hydrogen donating ability and thus the antioxidant activity of the free radical scavengers.

In our study, ZnO nanoparticles showed scavenging of DPPH radicals on different days, and percentage of scavenging increased linearly with the increase in concentration for ZnO nanoparticles and ascorbic acid (Fig. 2). There was also significant reduction in the scavenging of DPPH radicals by different concentrations of ZnO nanoparticles on day 30 and 60. The IC_{50} value of ZnO nanoparticles against DPPH radicals on day 1 was lesser as compared to day 30 and 60 values *i.e.*, 229.67±1.66 versus 323.53±1.21 and 381.45±0.86 (Table 1). This was might be due to agglomeration of particles on storage.

Superoxide anion radicals are oxygen centered radicals generated by the transfer of one electron and play an important role in the formation of other ROS such as hydroxyl radical, hydrogen peroxide, or singlet oxygen in living systems, which causes the lipid peroxidation (Stief 2003). Thus, superoxide anions radicals are considered as precursors to active free radicals that have potential for reactivity with biological molecules and there by inducing tissues damage (Ak and Gulcin 2008). Superoxides were dangerous to the body cell as they had

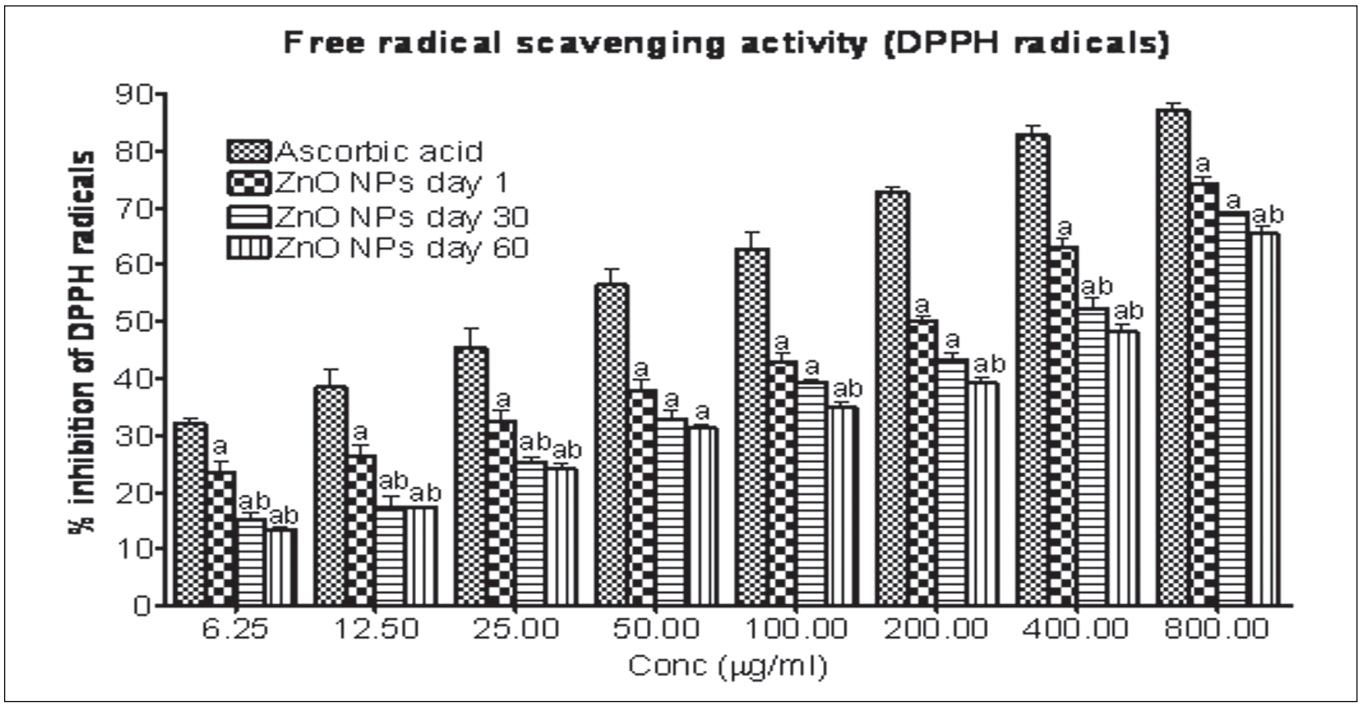

Fig. 2. Free radical scavenging activity (DPPH radicals scavenging) of different concentrations of ZnO nanoparticles (NPs) on days 1, 30 and 60.

Results are expressed as Mean ± SE (n = 3). [a, b,] represent $p < 0.05$ vs ascorbic acid and ZnO nanoparticles day 1, respectively at the same concentration.

the ability to oxidize DNA and protein (Robak and Gryglewsk 1988). In this study, ZnO nanoparticles scavenge the superoxide anion radicals markedly in concentration dependent manner (Fig. 3). On storage of suspension of ZnO nanoparticles for 30 and 60 days, significant reduction in the scavenging of superoxide anion radicals was also observed, which was might be due to agglomeration of particles on storage. The IC_{50} values (Table 1) of ZnO nanoparticles on day 1, 30 and 60 against superoxide anion radicals were 336.31±1.08, 383.85±1.39 and 429.37±1.13, respectively. This indicated that IC_{50} value of ZnO nanoparticles for superoxide anion radicals also increased significantly with its storage in suspension form due to agglomeration of particles.

Hydrogen peroxide assay give rise to hydroxyl radicals in cells, which itself is not very reactive but sometimes toxic to the cells (Halliwell 1991). Free radicals are scavenged by means of reduction. The reducing power of a compound is linked to the antioxidant activity and thus, the compounds with high reducing ability can exhibit potent free radical scavenging activity. In the present study, the reductive potential of nanoparticle was exhibited in concentration dependent manner (Fig. 4). It was evident from the graph that percentage of scavenging increased with increase in concentration of nanoparticles and ascorbic acid. There was also significant reduction in the scavenging of hydrogen peroxide radicals by different concentrations of ZnO nanoparticles on day 30 and 60. On day 1, the IC_{50} value of ZnO nanoparticles against hydrogen peroxide radicals was significantly lesser as compared to day 30 and 60 values (Table 1). Reduction in the scavenging of different free radicals by ZnO nanoparticles after its storage for 30 and 60 days was might be due to agglomerations of the nanoparticles during the storage, which increased nanoparticles size and decreased actions of the nanoparticles (Bruinink *et al.* 2015, Zakharova *et al.* 2015). It was also evident for all the tested free radicals that their scavenging was better for ascorbic acid (standard antioxidant) as compared to ZnO nanoparticles at all the tested concentrations (Fig. 1-4). The IC_{50} values of ascorbic acid were also significantly lower than ZnO nanoparticles against all tested free radicals (Table 1). The IC_{50} represent the concentration (µg/ml) of ZnO nanoparticles or ascorbic acid required for the 50% inhibition of different radicals. The IC_{50} values for different radicals were calculated by using the linear regression analysis. Lower IC_{50} value of a compound shows its more potency.

The values of MIC and MBC for the synthesized ZnO nanoparticles, tetracycline and gentamicin against *S. aureus* and *E. coli* bacterial strains are presented in

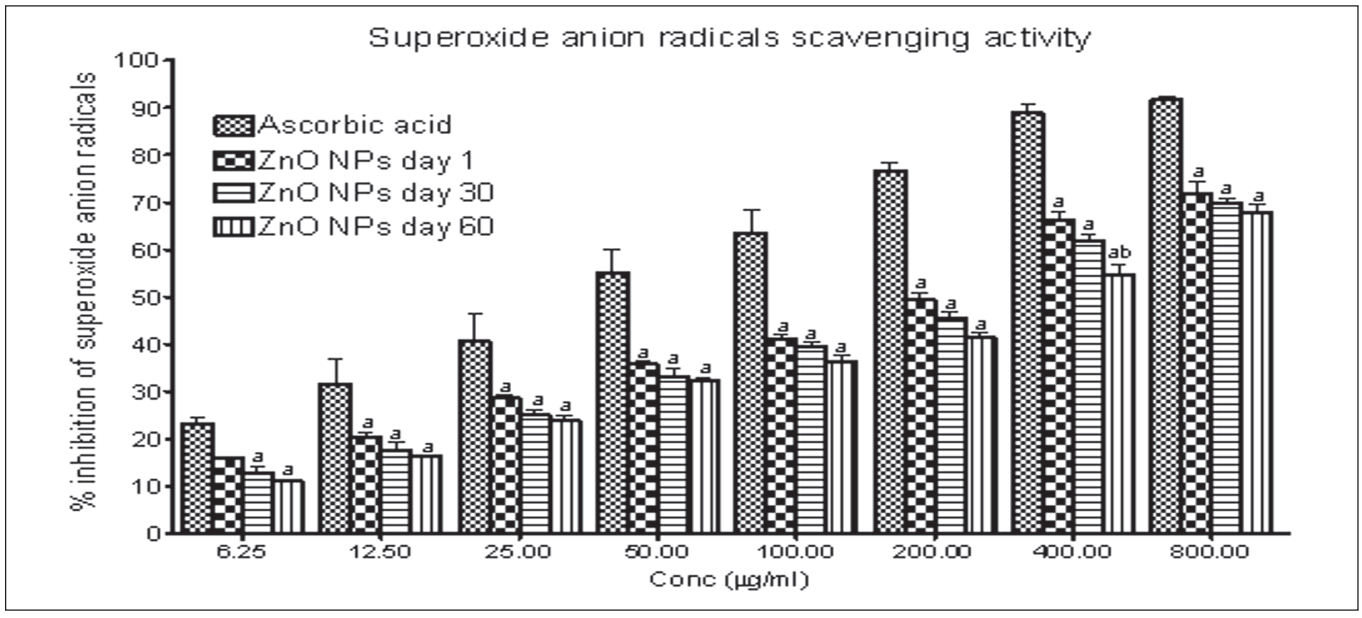

Fig. 3. Superoxide anion radicals scavenging ability of different concentrations of ZnO nanoparticles (NPs) on days 1, 30 and 60.

Results are expressed as Mean ± SE (n = 3). [a] represents $p < 0.05$ vs ascorbic acid at the same concentration.

Table 2. The ZnO nanoparticles showed antibacterial actions against both the bacterial strains which revealed its powerful broad spectrum anti-bacterial activity. The values of MIC and MBC of ZnO nanoparticles were lower for *S. aureus* than *E. coli* and this revealed that their action was more against *S. aureus* than *E. coli*. Morphological difference, particularly in cell wall and cell membranes, among the two bacterial strains might attribute the disparity in their sensitivity and leads to the difference in resistance against intrusion of nanoparticle through the walls of bacteria (Stensberg *et al.* 2011, Bruslind 2017, Wang *et al.* 2017). Physiochemical characteristics of ZnO nanoparticles such as size, shape, chemical composition and solubility are also associated with efficacy of ZnO

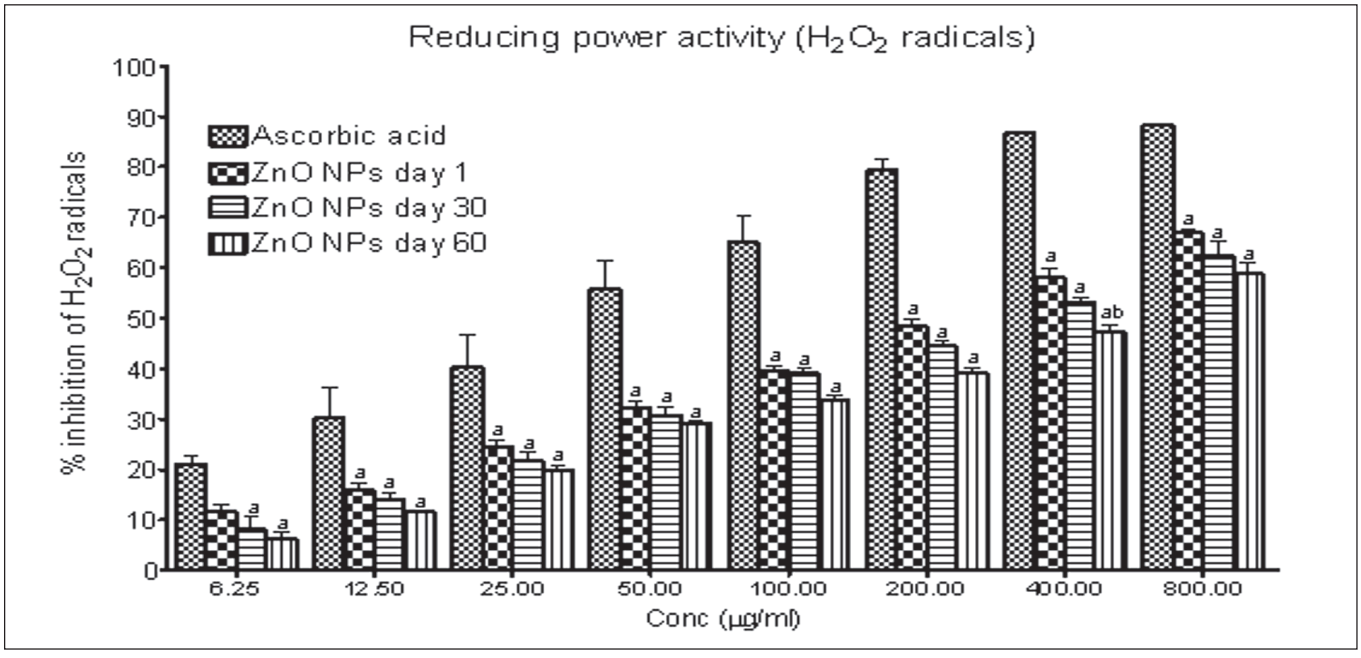

Fig. 4. Reducing power activity (H_2O_2 radicals scavenging) of different concentrations of ZnO nanoparticles (NPs) on days 1, 30 and 60.

Results are expressed as Mean ± SE (n = 3). [a] represents $p < 0.05$ vs ascorbic acid at the same concentration.

nanoparticles (Yambo and Feyissa 2013). In present study also, the size of ZnO nanoparticles affect their efficacy. It was evident in present study that MIC and MBC values for *S. aureus* and MBC value for *E. coli* were increased after storage of ZnO nanoparticles for two months. This increase in MIC and MBC value after storage of suspensions of ZnO nanoparticles was might be due to its increased size of nanoparticles due to the agglomerations of nanoparticles during the storage, which resulted in decreased actions of the nanoparticles (Yambo and Feyissa 2013, Bruinink *et al.* 2015, Zakharova *et al.* 2015). The same MIC value for *E. coli* on day 1, 30 and 60 was might be due to the reason that the MIC value falls between 16 to 32 µg/ml. It is well evident that large surface area property of nanoparticles leads to their tightly adsorption on the surface of the bacterial cells, which impairs the bacterial membrane permeability leading to the leakage of intracellular components and thus killing the bacterial cells (Qi *et al*. 2004). It has been showed that the small size of the nanoparticles may have a greater impact on their bioactivity due to greater accumulation of the nanoparticles inside the cell membrane and cytoplasm (Venckatesh *et al*. 2013, Jones *et al.* 2008). Earlier reports of ZnO nanoparticles have revealed that the smaller the particle size, the greater the efficacy in inhibiting the growth of bacteria (Raghupathi *et al.* 2011). ZnO nanoparticles with average size of 12 nm (concentration of 3mM) can cause 100% inhibition of bacterial growth due to impairment of cell membrane permeability of *E. coli* leading to the accumulation of nanoparticles in the bacterial membrane and cytoplasm regions of the cells (Brayner *et al.* 2006). The exact mechanisms of antibacterial actions by ZnO nanoparticles are still not clear, but different studies have proposed that several mechanisms are associated with its antimicrobial activity. Some of the mechanisms include metal ion release (Nagy *et al.* 2011), oxidative stress induction (Gurunathan *et al.* 2012), non-oxidative stress (Leung *et al.* 2014). Earlier studies have also shown that ZnO nanoparticles possess broad antibacterial potentials against different types of gram positive and negative bacteria (Espitia *et al.* 2012).

CONCLUSION

In conclusion, ZnO nanoparticles synthesized in this study showed antioxidant activities (against ABTS, DPPH, superoxide anion and hydrogen peroxide radicals) and antibacterial potentials. These properties of ZnO nanoparticles may be extended in the management of different diseases associated with free radicals and microorganisms after testing its efficacy in experimental laboratory animals. Moreover, in future, further detailed studies to explore the mechanisms of actions for these potentials and safety evaluation studies on eukaryotic cells are needed for the commercialization of these nanoparticles in different fields like Veterinary sciences, Medical sciences, Food sciences, Agriculture etc.

ACKNOWLEDGEMENT

We are thankful to Department of Chemistry and Department of Biotechnology, University of Jammu (J&K), for providing essential facilities and support for conducting present investigation. The first author is highly thankful to CSIR UGC, New Delhi, India for providing financial support in the form of Junior Research Fellowship (JRF).

REFERENCES

Ak T, Gulcin I (2008) Antioxidant and radical scavenging properties of curcumin. Chem Biol Interact 174(1): 27–37.

Ankanna S, Savithramma N (2011) Biological synthesis of silver nanoparticles by using stem of *Shorea tumbuggaia* Roxb. and its antimicrobial efficacy. Asian J Pharm Clin Res 4: 137-141.

Bala N, Saha S, Chakraborty M, Maiti M, Das S *et al.* (2015) Green synthesis of zinc oxide nanoparticles using *Hibiscus subdariffa* leaf extract: effect of temperature on synthesis, anti-bacterial activity and anti-diabetic activity. RSC Adv 5(7): 4993-5003.

Bhakya S, Muthukrishnan S, Sukumaran M, Muthukumar M (2015) Biogenic synthesis of silver nanoparticles and their antioxidant and antibacterial activity. Appl Nanosci 10: 01-12.

Brayner R, Ferrari-Iliou R, Brivois N, Djediat S, Benedetti MF *et al.* (2006) Toxicological impact studies based on *Escherichia coli* bacteria in ultrafine ZnO nanoparticles colloidal medium. Nano Lett 6(4): 866-870.

Bruinink A, Wang J, Wick P (2015) Effect of particle agglomeration in nanotoxicology. Arch Toxicol 89: 659-675.

Bruslind L (2017) Bacteria: Internal components. In Microbiology. Open Oregon Stat, Corvallis, OR, USA.

Cho K, Wang X, Nie S, Shin DM (2008) Therapeutic nanoparticles for drug delivery in cancer. Clin Cancer Res 14: 1310-1316.

CLSI (2012) Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically, approved standard, 9th edn. CLSI document M07-A9. Clinical and

Laboratory Standards Institute, 950 West Valley Road, Pennsylvania 19087, USA.

Contreras-Guzman ES, Strong FC (1982) Determination of tocopherols (Vitamin E) by reduction of cupricion. J AOAC Int 65: 1215-1217.

Espitia PJP, Soares NDFF, dos Reis Coimbra JS, de Andrade NJ, Cruz RS *et al.* (2012) Zinc oxide nanoparticles: synthesis, antimicrobial activity and food packaging applications. Food Bioprocess Technol 5(5): 1447-1464.

Ewnetu Y, Lemma W, Birhane N (2013) Antibacterial effects of *Apis mellifera* and stingless bees' honeys on susceptible and resistant strains of *Escherichia coli*, *Staphylococcus aureus* and *Klebsiella pneumoniae* in Gondar, Northwest Ethiopia. BMC Complement Altern Med 13: 269.

Gulcin I, Kufrevioglu OI, Oktay M, Buyukokuroglu ME (2004) Antioxidant, antimicrobial, antiulcer and analgesic activities of nettle (*Urtica dioica* L.). J Ethnopharmacol 90(2-3): 205-215.

Gupta V, Magotra U, Sandarve, Sharma AK, Sharma M (2016) A study of concentration and temperature dependent effect on speed of sound and acoustical parameters in zinc oxide. Nanofluid Sci Int 4(2): 39-50.

Gurunathan S, Han JW, Dayem AA, Eppakayala V, Kim JH (2012) Oxidative stress-mediated antibacterial activity of graphene oxide and reduced grapheme oxide in *Pseudomonas aeruginosa*. Int J Nanomedicine 7: 5901-5914.

Halliwell B (1991) Reactive oxygen species in living systems: source, biochemistry, and role in human disease. Am J Med 91: 14-22.

Hsu B, Coupar IM, Ng K (2006) Antioxidant activity of hot water extract from the fruit of the Doum palm, *Hyphaene thebaica*. Food Chem 98(2): 317-328.

Huang D, Ou B, Prior RL (2005) The chemistry behind antioxidant capacity assays. J Agric Food Chem 53(6): 1841-1856.

Humberto HL, Ayala-Nunez NV, Liliana del Carmen IT, Cristina RP (2010) Bactericidal effect of silver nanoparticles against multidrug-resistant bacteria. World J Microbiol Biotechnol 26: 615-621.

Ilhami G, Zubeyrn H, Mahfuz E, Hassan Y, Aboul E (2010) Radical scavenging and antioxidant activity of tannic acid. Arab J Chem 3(1): 43-53.

Jamdagni P, Khatri P, Rana JS (2018) Green synthesis of zinc oxide nanoparticles using flower extract of *Nyctanthes arbor-tristis* and their antifungal activity. J King Saud Univ Sci 30: 168-175.

Jones N, Ray B, Ranjit KT, Manna AC (2008) Antibacterial activity of ZnO nanoparticle suspensions on a broad spectrum of microorganisms. FEMS Microbiol Lett 279(1): 71-76.

Kant V, Mehta M, Varshneya C (2012) Antioxidant potential and total phenolic contents of seabuck thorn (*Hippophae rhamnoides*) pomace. Free Radic Antioxid 2(4): 79-86.

Ko KS, Koh DC, Kong IC (2018) Toxicity evaluation of individual and mixtures of nano-particles based on algal chlorophyll content and cell count. Materials 11(1): 121-125.

Leung YH, Ng AMC, Xu X, Shen Z, Gethings LA *et al.* (2014) Mechanisms of antibacterial activity of MgO: non-ROS mediated toxicity of MgO nanoparticles towards *Escherichia coli*. Small 10(6): 1171-1183.

Nagy A, Harrison A, Sabbani S, Munson RS, Dutta PK *et al.* (2011) Silver nanoparticles embedded in zeolite membranes: release of silver ions and mechanism of antibacterial action. Int J Nanomedicine 6: 1833-1852.

Nash KM, Ahmed S (2015) Nanomedicine in the ROS-mediated pathophysiology: applications and clinical advances. Nanomedicine 11(8): 2033-2040.

Nishikimi M, Rao NA, Yagi K (1972) The occurrence of superoxide anion in the reaction of reduced phenazine methosulphate and molecular oxygen. Biochem Biophys Res Commun 46(2): 849-853.

Nita M, Grzybowski A (2016) The role of the reactive oxygen species and oxidative stress in the patho-mechanism of the age-related ocular diseases and other pathologies of the anterior and posterior eye segments in adults. Oxid Med Cell Longev 2016: 3164734.

Panda K, Golari D, Venugopal A, Achary V, Phaomei G *et al.* (2017) Green synthesized zinc oxide (ZnO) nanoparticles induce oxidative stress and DNA damage in *Lathyrus sativus* L. root bioassay system. Antioxidants 6(2): 35.

Pati R, Mehta RK, Mohanty S, Padhi A, Sengupta M *et al.* (2014) Topical application of zinc oxide nanoparticles reduces bacterial skin infection in mice and exhibits antibacterial activity by inducing oxidative stress response and cell membrane disintegration in macrophages. Nanomedicine 10: 1195-1208.

Qi L, Xu Z, Tiang X, Hu C, Zou X (2004) Preparation and antibacterial activity of chitosan nanoparticles. Carbohyd Res 339: 2693-2700.

Raghupathi KR, Koodali RT, Manna AC (2011) Size-dependent bacterial growth inhibition and mechanism of antibacterial activity of zinc oxide nanoparticles. Langmuir 27(7): 4020-4028.

RatneyYJJJ, David SB (2017) Evaluation of *in vitro* anticancer and antioxidant activity of zinc oxide nanoparticle by chemical and green method. IJETT Special Issue: 10-15.

Re R, Pellegrini N, Proteggente A, Pannala A, Yang M *et al.* (1999) Antioxidant activity applying an improved ABTS radical cation decolorization assay. Free Radic Biol Med 26(9-10): 1231-1237.

Rehana D, Senthil DMR, Kalilur KA (2017) *In vitro* antioxidant and antidiabetic activities of zinc oxide nanoparticles synthesized using different plant extracts. Bioprocess Biosyst Eng 40(6): 943-957.

Robak J, Gryglewsk RJ (1988) Flavonoids are scavenger of superoxide anions. Bio Chem Pharm 37(5): 837-841.

Sangeetha G, Rajeshwari S, Venckatesh R (2011) Green synthesis of zinc oxide nanoparticles by *Aloe barbadensis* Miller leaf extract: structure and optical properties. Mater Res Bull 46: 2560-2566.

Singh BN, Kumar A, Rawat S, Khan W, Naqvi AH *et al.* (2014) Biosynthesis of stable antioxidant ZnO nanoparticles by *Pseudomonas aeruginosa* rhamnolipids. PLoS One 9(9): e106937.

Stensberg MC, Wei Q, McLamore ES, Porterfield DM, Wei A *et al.* (2011) Toxicological studies on silver nanoparticles: challenges and opportunities in assessment, monitoring and imaging. Nanomed 6: 879-898.

Stief TW (2003) The physiology and pharmacology of singlet oxygen. Med Hypoth 60(4): 567–572.

Tettey CO, Shin HM (2019) Evaluation of the antioxidant and cytotoxic activities of zinc oxide nanoparticles synthesized using *Scutellaria baicalensis* root. Sci Afr 6: 00157.

Velavan S (2011) Free radicals in health and diseases - a mini review. Pharmacol News lett 1: 1062-1077.

Venckatesh R, Rajiv P, Rajeshwari S (2013) Bio-fabrication of zinc oxide nanoparticles using leaf extract of *Parthenium hysterophorus* L. and its size-dependent antifungal activity against plant fungal pathogens. Spectrochim Acta A Mol Biomol Spectrosc 112: 384-387.

Vijayakumar S, Vaseeharan B, Malaikozhundan B (2016) *Laurus nobilis* leaf extract mediated green synthesis of ZnO nanoparticles: characterization and biomedical applications. Biomed Pharmacother 84: 1213-1222.

Wang L, Hu C, Shao L (2017) The antimicrobial activity of nanoparticles: present situation and prospects for the future. Int J Nanomed 12: 1227-1249.

Yambo Y, Feyissa T (2013) Micropropagation of anchote [*Coccinia abyssinica* (Lam.) Cogn.]: high calcium content tuber crop of Ethiopia. Afr J Agric Res 8(46): 5915-5922.

Yaqoob AA, Ahmad H, Parveen T, Ahmad A, Oves M *et al.* (2020) Recent advances in metal decorated nanomaterials and their various biological applications: a review. Front Chem 8: 341.

Zakharova OV, Godymchuk AY, Gusev AA, Gulchenko SI, Vasyukova IA *et al.* (2015) Considerable variation of antibacterial activity of Cu nanoparticles suspensions depending on the storage time, dispersive medium, and marticle sizes. BioMed Res Int. Article ID 412530.

***Cite this article as:** Gupta V, Kant V, Sharma M (2020) Synthesis and *in vitro* assessment of zinc oxide nanoparticles for their antioxidant and antibacterial potentials. Explor Anim Med Res 10(2): 124-133.